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SEPTORIA CANKER of POPLARS in the UNITED STATES

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SEPTORIA CANKER of POPLARS in the United States

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INTRODUCTION

During the past few years the hybridization of poplars has received considerable attention, both in America and Europe, in an attempt to produce rapidly growing trees that would attain merchantable size in a relatively short period. Such trees would be suitable for pulpwood, veneer, matches, and other wood products. The hybrid trees would be useful also for shelterbelts, windbreaks, reforestation, and in soil conservation along river banks. Many hybrid poplar trees have been attacked by fungus diseases that have caused their death within a few years. For this reason, disease resistance is important in the poplar breeding program.

A serious disease of hybrid poplars in the United States and Canada at the present time is that caused by the fungus *Septoria musiva* Peck; this is a common leaf-infecting fungus on native poplars, but it produces cankers as well as leaf spots on some exotic species and on hybrid poplars, particularly those with black, balsam, and cottonwood parentage. Since the first report of the canker in the United States (*21*)[2] and the isolation of the causal organism, a study has been made of the manner in which infection occurs in nature, the action of the fungus upon the hosts, and the relative susceptibility of selected hybrid poplar clones that represent crosses between various species of poplar. The rating of susceptibility was based upon the reaction of the hosts following artificial inoculation.

The immediate occasion for a detailed study of the disease was the extensive injury to hybrid poplar clones in plantations in the eastern United States. These clones had been selected from hybrid seedlings obtained by Stout and Schreiner (*19, p. 1276*) from crosses with 34 poplar species and hybrids. The parent trees were single trees of species, varieties, or hybrids growing at Highland Park, Rochester, N. Y. Each parent species, variety, or hybrid was represented by a male or a female tree but not by both sexes, except in *Populus deltoides*

[1] Acknowledgment is made to E. J. Schreiner of the Northeastern Forest Experiment Station for assistance in all phases of the study, and to Kenneth F. Aldrich, biological science aid, Division of Forest Disease Research, for assistance in making inoculations, collecting data, and recording results.

[2] Italic numbers in parentheses refer to Literature Cited, p. 23.

Marsh., × *P. berolinensis* Dipp., *P. sargentii* Dode, and *P. simonii* Carr.

The species or variety names of the parents of the hybrid seedlings tested in this study are those by which they were designated in the original records at Highland Park, Rochester, N. Y., and are as follows, with authorities included, so far as known, for the most part according to Rehder's Manual of Cultivated Trees and Shrubs, second edition: *P. angulata* Ait., × *P. berolinensis* Dipp., *P. berolinensis rossica*, *P. caudina* Tenore, *P. candicans* Ait., *P. charkoviensis* Schroed., *P. deltoides* Marsh., *P. eugenei* Simon-Louis, *P. fremontii* S. Wats., *P. grandidentata* Michx., *P. incrassata* Dode, *P. laurifolia* Ledeb., *P. maximowiczii* Henry, *P. nigra* L., *P. nigra betulifolia* (Pursh) Torr., *P. nigra bautanicorum ritrum*, *P. nigra italica* Muenchh., *P. nigra plantierensis* (Simon-Louis) Schneid., × *P. petrowskyana* Schneid., × *P. rasumowskyana* Schneid., × *P. robusta* Schneid., *P. sargentii* Dode, × *P. serotina* Hartig, *P. simonii* Carr., *P. trichocarpa* Hook., *P. volga*.

A plantation of some 13,000 hybrid seedlings developed in this breeding project was established in 1924 at Frye, Maine, by the Oxford Paper Company, in cooperation with the New York Botanical Garden. In 1936 the plantation was transferred to the Northeastern Forest Experiment Station of the U. S. Forest Service. Several plantings of selected hybrid clones were made also in various localities throughout the United States, to test the response of the clones to various soil and climatic conditions.

Septoria canker has caused extensive losses, particularly in hybrid poplar plantings in Massachusetts, Vermont, New York, Maine, and Maryland, and in those established by the Tennessee Valley Authority at Norris, Tenn. (*3*). It occurs also in recent plantings in Wisconsin and Minnesota. Clonal variation in susceptibility has been evident, suggesting the possibility that, by carefully selecting hybrid clones that resist natural infection and by testing susceptibility by means of artificial inoculations, a group of resistant and desirable hybrid poplar clones might be obtained.

A series of clones of hybrids, species, and varieties of poplars[3] selected by Pauley from the collection of clones developed by Harvard University under the Cabot Foundation for Botanical Research (*14*) was established at the Arnold Arboretum, Jamaica Plain, Mass., for the purpose of testing disease resistance. These clones were inoculated with *Septoria musiva* and rated for susceptibility in connection with the present study.

HISTORY OF THE DISEASE

Early reports from the Northern Great Plains Field Station, U. S. Department of Agriculture, at Mandan, N. Dak. (*10*, *24*), stated that in experimental tests of various species of trees for use in shelterbelts, poplars designated as "Northwest" and "Canadian" were subject to canker. The Northwest poplar was considered to be a natural hybrid of unknown parentage, possibly of *Populus tacamahaca* Mill., crossed with *P. deltoides*, originating in the north-central part of North Dakota. The so-called Canadian poplar (*24*) was reported to be one of the intro-

[3] Hybrids, species, and varieties in this series that are not included in the list given in column 1 are as follows: × *P. generosa* Henry, × *P. jackii* Sarg., *P. korcana* Rehd., *P. trichocarpa hastata* (Dode) Henry.

duced hybrids propagated by commercial nurseries in the United States and Canada.

In 1923 Johnson and Cobb (*13*) reported the canker as "a disease which soon girdles the tree, generally near the base or at a crotch, and kills the portion above the canker," but its cause was not determined. In Canada (*7*), a similar disease became severe on poplars in Saskatchewan, and in 1928 a study of the cause was begun at Indian Head, Sask. (*8*). Among the fungi isolated from the cankers was a species of *Septoria* which, when inoculated into healthy plants in the greenhouse, reproduced the disease symptoms.

In 1939 Bier (*1*) described in detail the causal organism and its effect on introduced hybrid poplars and on native hybrids at Indian Head and at Petawawa Forest Experiment Station, Ontario. He identified the causal organism as *Septoria musiva*, indigenous in North America, and originally described on living leaves of *P. deltoides* in Albany, N. Y. (*15, p. 138*). His inoculations of cuttings in the greenhouse proved that Northwest poplar was highly susceptible to stem infection and that Saskatchewan poplar, a native hybrid of similar parentage, was slightly susceptible. Inoculations of cuttings of *P. tacamahaca* and *P. deltoides*, however, indicated that these species, the probable parents of the hybrids, were resistant. *P. rasumowskyana*, *P. petrowskyana* and *P. berolinensis*, the introduced hybrids that had been extensively planted in Canada were highly susceptible when artificially inoculated. All poplars tested by Bier, including the native species, were susceptible to leaf infection under natural conditions, as well as by inoculation.

Recently Sarasola (*17*) described a canker of poplars in Argentina; he reported the causal organism to be *S. musiva*, which also infected the leaves. The fungus was first observed in that country in 1941 and was particularly serious on several hybrid poplars that had been introduced from Europe and the United States. The origin of the fungus was not known.

SYMPTOMS

Because of the wide variation in host reaction to stem infection among the different hybrid poplar clones, no distinctive canker can be described as definitely peculiar to septoria infection. Also the rapid invasion of infected areas by secondary fungi tends to mask the presence of *Septoria musiva*, the primary organism. The presence of pycnidia of *S. musiva* on the cankers or the isolation of the fungus in cultures from wood tissue are the only means by which an exact diagnosis can be made. Infection of stems is preceded by leaf infection, which occurs early in the season on the leaves of young shoots or low branches. Older trees with no basal shoots and with the lowest branches some distance from the ground may have little or no leaf infection even though the branches or stems are already infected.

In a planting of hybrid poplar clones newly established from unrooted cuttings, leaf infection may occur during the first season from *Septoria* spores that lodge in the lenticels and in the resinous coating of the buds of dormant cuttings that have been obtained from infected stool beds. These spores remain viable during storage (*22*) and may germinate when conditions become favorable. Early infection may be caused also by windborne spores

from pycnidia in leaves of nearby native poplars. In established plantings, leaves on young basal shoots or those on the lowest branches are most commonly infected and leaf spots may appear soon after the leaves develop (fig. 1). A high percentage of this infection probably is caused by the ascospores of the perfect stage, *Mycosphaerella populorum* G. E. Thompson discharged from perithecia in overwintered leaves on the ground. Under favorable moisture conditions the leaf spots increase rapidly in size and number. Pink spore tendrils exude from small pycnidia in the leaf spots and the spores are washed onto the bark of stems or twigs.

FIGURE 1.—Leaf spots caused by *Septoria musiva*.

Young vigorously growing shoots and stems may become infected around the base of a diseased leaf, or through lenticels (fig. 2), or small abrasions in the bark.

FIGURE 2.—Canker on stem of hybrid poplar (*Populus nigra* × *P. laurifolia*) resulting from natural infection by *Septoria musiva*.

The affected area becomes blackened and slightly depressed. As the tissue dries, it turns dark brown, sometimes forming a lesion with black margin, light tan at the center with a few inconspicuous brown pycnidia from which *Septoria* spores exude in moist weather. These pycnidia are usually formed about 4 weeks after infection. During spring rains the exuding spores may be washed down the stem or onto the leaves, causing new infections. A series of lesions may thus be formed along

the stem around infected lenticels. The period of fruiting is usually very brief and the fertile hyphae seem to be limited to a small area of the lesion.

Later a few obscure pycnidia may be formed in the lenticels of the lesion, but apparently the fungus soon develops a vegetative stage by which its advance in the bark is continued. The spread of the mycelium is preceded by a blackening of the cambium. In sections through the discolored cambium no hyphae have been found and wood tissue placed on malt agar has remained sterile.

On the stems of highly susceptible hosts, the newly affected area appears slightly sunken, with smooth bark and raised margin. The mycelium gradually spreads beyond this margin and usually, before the end of the growing season, produces a canker with several slightly raised irregularly concentric rings of unbroken bark. These raised rings, on both young and old cankers, are similar to those on cankers caused by *Cytospora chrysosperma* Pers. ex Fr. and *Nectria* species on aspen and other native and hybrid poplars. These latter rings, however, are more regular and their causal fungi produce abundant and conspicuous fruiting bodies, thus differing from the septoria cankers with smooth bark, irregular rings of raised bark, and few or no *Septoria* pycnidia visible to the naked eye.

Stems less than three-fourths inch in diameter may be girdled by *Septoria musiva* in one growing season. On larger stems, particularly those of vigorously growing but highly susceptible hosts, young cankers may have at the center a thin layer of brown, varnishlike, brittle exudate covering the lenticel, in which infection took place. This is probably a host reaction to infection and large *Septoria* pycnidia may be found in or around this area. As the cankers increase in size, the wood may be killed inward to the pith, producing a flat-faced canker (fig. 3), swollen at the sides, and

FIGURE 3.—Depressed flat-faced cankers on stems of hybrid poplars, resulting from inoculations with *Septoria musiva*. Left, *Populus charkoviensis* × *P. caudina*; right, *P. angulata* × *P. trichocarpa*.

distorting the stem (fig. 4). These cankers usually are perennial and increase longitudinally for several years. Eventually an infected trunk may become so weakened at the canker that it breaks in a windstorm (fig. 5). Frequently the rapid growth of the stem tissue prevents the girdling of the stem, and the tree may live for several years with one or more disfiguring but nongirdling cankers.

Vigorous shoots developed from cuttings, or from stumps of trees in stool beds or of trees that have been cut in thinning operations, are most readily infected when they are about 2 feet in height. In a close planting, the small stem lesions are

FIGURE 4.—Canker on stem of hybrid poplar (*Populus maximowiczii* × *P. nigra plantierensis*) from inoculation with *Septoria musiva*, showing distortion of living stem.

FIGURE 5.—Stem of hybrid poplar (*Populus maximowiczii* × *P. nigra plantierensis*) broken at canker resulting from inoculation with *Septoria musiva*.

obscured by the dense foliage. The high humidity near the ground is especially favorable for the development of serious basal cankers. In plantings where the trees are widely spaced and humidity is thus reduced, the stems may escape infection during their first few years of growth, even though they are of highly susceptible clones with abundant leaf spots. Thereafter they may remain free from stem infection or may develop high trunk cankers from infection of the new terminal growth. These, as well as the basal cankers, may escape detection until they have girdled the trunks.

Adventitious shoots may be produced below the trunk cankers, particularly near the base of the tree, and these will readily become infected. On basal cankers where humidity is high, *Septoria* pycnidia are frequently found in the lenticels. Spores are produced in them throughout the growing season and even the following spring, thus providing inoculum for early spring infection. The new growth of twigs or branches of older and highly susceptible trees is subject to infection, but the resulting lesions or cankers cause less injury than the trunk cankers.

Stems infected by *Septoria musiva* are rapidly attacked by secondary fungi. On cankers of all sizes fruiting bodies of various perfect and imperfect fungi may be found. The prevalence of these fruiting bodies in contrast to the scarcity of *Septoria* pycnidia may easily lead to an incorrect diagnosis. In order to determine definitely whether a canker was caused by *S. musiva*, it is necessary to find the pycnidia on the canker, particularly in the lenticels, or to isolate the fungus from the wood at the advancing edge of the canker. Cultures from wood tissue frequently

develop only the vegetative mycelium of the *Septoria* and this makes it more difficult to identify the causal organism. From old trunk cankers it may be impossible to isolate the *Septoria* and to determine the causal organism.

On the more resistant hosts, lesions begin development in the same way as they do on the susceptible hosts, but the mycelium of the *Septoria* advances very slowly. It may cease growth after the current growing season and the small lesion will become healed. Frequently the bark of the lesion cracks at the center soon after infection and the production of callus begins. The wood at the point of infection becomes exposed, with thick rolls of callus at each side (fig. 6). On the stems of some clones, the callus eventually heals over the exposed wood. On less resistant clones the wood becomes exposed more widely and infection by wood-rotting fungi occurs. It is impossible to isolate the *Septoria* from the wood tissue of such cankers and no pycnidia of the fungus are formed. These cankers, therefore, cannot be distinguished from similar cankers caused by other fungi.

FIGURE 6.—Canker on stem of hybrid poplar (*Populus petrowskyana* × *P. candina*), resulting from natural infection by *Septoria musiva*, showing development of callus. Cankers of this type on poplars may be caused by other fungi.

HOSTS AND DISTRIBUTION

Septoria musiva has been reported as a leaf-infecting fungus on all species of aspens and poplars native in the United States and its distribution is widespread. Thompson (*20*) proved by the inoculation of cuttings in the greenhouse that the leaves of 26 species and varieties of native and exotic poplars are susceptible to infection. He made no stem inoculations but twig infection developed on one shoot of *Populus szechuanica* Schneid. From inoculations in the greenhouse Bier (*1*) obtained stem lesions on several hybrid poplars but found that *P. tacamahaca* and *P. deltoides*, the parents of two of the hybrids, were resistant to canker formation. These species, as well as the hybrids tested, were susceptible to leaf infection. No references have been found in literature to septoria cankers on any native species. In the summer of 1951 roadside poplar trees at New Hartford, Conn., and at Great Barrington, Mass., were found to have considerable leaf infection, and, on small twigs, cankers on the current year's growth bore mature *Septoria* fruiting bodies. These trees may be natural hybrids. In both localities the trees were situated in moist shady areas particularly favorable for infection. On older twigs a few small heavily calloused cankers were found but these could not be definitely diagnosed as sep-

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toria cankers. No branch or trunk cankers were found.

In Canada (*1*) the hybrid poplars *P. rasumowskyana*, *P. petrowskyana*, and *P. berolinensis*, as well as the native hybrids, Northwest poplar and Saskatchewan poplar, were found to be susceptible to the disease under natural conditions.

The writer found that, under natural conditions in the eastern United States, a few trees of *P. maximowiczii* developed cankers that were perennial for several years, but the trees are still maintaining a good yearly growth. On one such tree, started as a cutting in 1916, an open canker extended from the ground level upward about 5 feet, with several successive rolls of callous tissue at the sides. From the inner bark and wood at the upper margin of the canker, cultures typical of *S. musiva* were obtained in 1949. From smaller trunk cankers on trees of *P. maximowiczii* in another locality, similar cultures from the marginal wood also produced mature *Septoria* fruiting bodies. This indicates that, although certain trees of *P. maximowiczii* may be severely infected by *S. musiva* when young, their vigorous growth is sufficient to offset the effects of the fungus. High trunk cankers on a few of the trees resulted in breakage during windstorms. Leaf spots and occasional small twig cankers with sporulating pycnidia of the fungus were present on all infected trees of this species: such trees may be considered a continual source of inoculum for spreading the disease.

So far as is known at the present time, species of poplar native in the United States are highly resistant to stem infection by *S. musiva*. However, no detailed study of the relative resistance of clones of species trees, tested by means of artificial inoculation, has yet been undertaken. Observations indicate that, with the possible exception of *P. alba* L. and *P. canescens* (Ait.) Sm., no species or hybrid of poplar can be considered as immune, since all native species and a large number of European and Asiatic species are susceptible to leaf infection. Therefore, hybrids grown anywhere within the range of our native poplars may prove susceptible to *S. musiva* as a canker-producing fungus.

THE CAUSAL ORGANISM

The fungus isolated from leaves and cankers on hybrid poplars in the United States is morphologically the same as the one originally described as *Septoria musiva* Peck (*15*, *p. 138*). Its morphology and cultural characteristics correspond also with those described for *S. musiva* by Bier (*1*), Thompson (*20*), and Sarasola (*17*). Davis (*4*) collected leaf spots on *Populus tacamahaca*, *P. deltoides*, *P. tremuloides* Michx., and *P. grandidentata*, all of which had pycnidia and spores of a *Septoria* that he considered to be *S. musiva*. He described five types of spots, differing in color, shape, and size, but found that the characteristics of the groups merged into one another so that no definite separation could be made. The pycnidia occurred on either or both leaf surfaces, and were distinct and innate, or almost superficial and collapsing. The conidia varied in septation and length, ranging from 3 to 6 septate and 18μ–70μ. Thompson's descriptions of the leaf spots, pycnidia, and conidia resulting from his inoculations of 26 species, varieties, and hybrids of poplar (*20*) suggest similar variations. In the present study, the characteristics of leaf spots, pycnidia, and conidia differed slightly among the hosts, and also according to time of infection and texture of the leaves

infected, and whether they were juvenile leaves on young succulent shoots or leaves on well-established trees.

Davis (*5*) also reported a collection of leaf spots on a species of poplar, "perhaps *Populus nigra*," caused by a species of *Septoria* with uni- or bi-septate conidia. He referred a specimen of this collection to Dr. F. Petrak, who identified it as *Septoria populi* Desm. and who considered *S. musiva* to be a form of that species. Green (*11*, *12*) identified the cause of leaf spots on hybrid poplar clones in Wisconsin as *S. populi*, and stated that, on the basis of Petrak's identification, Davis thereafter listed under *S. populi* all of his herbarium specimens previously reported as *S. musiva*.

In Argentina, Sarasola (*17*) reported that, since 1933, *S. populi* has been known as the cause of a leaf spot of *P. nigra italica*. The species was described by Desmazieres (*6*) in 1843 on *P. nigra* L. in France and has been reported on that host throughout Europe. Sarasola found that it differed from *S. musiva* in the appearance of the leaf spots, size and septation of the conidia, and cultural characteristics. Moreover, in his inoculations, infection by *S. populi* occurred only on leaves of an evergreen form [4] of *P. nigra italica*. Sarasola stated that the growth characteristics of *S. populi* were more definite and constant than those of *S. musiva*.

Thompson (*20*) reported another species, *Septoria populicola* Peck, on poplar leaf spots; this species was originally found in 1886 on living leaves of *P. balsamifera* L. at Keene, N. Y. (*16*, *p. 61*). Spores of *S. populicola* are consistently longer than those of *S. musiva*. In inoculations, Thompson found that *S. populicola* would infect leaves of *P. tacamahaca*, *P. trichocarpa*, and *P. szechuanica*, but failed to infect 21 other species and varieties of poplar.

Neither *S. populi* nor *S. populicola* has been reported as causing twig or stem cankers in nature or by inoculations. Therefore, it seems advisable at the present time to use the specific name *S. musiva* for the fungus causing the cankers on hybrid poplars and the common leaf spots on hybrid, exotic, and native poplars in the United States. Perfect stages of the three species of *Septoria* have been reported as *Mycosphaerella populi* (Auersw.) Schroet., *M. populicola* G. E. Thompson, and *M. populorum*, respectively, occurring on fallen leaves. The descriptions of these species also are similar, but the size of asci and ascospores is reported as showing distinct and consistent ranges that have been considered sufficient to separate the three species. The species of *Mycosphaerella* found on fallen leaves of the hybrid clones in the present study corresponds with the description and type specimen of *M. populorum*.

The pycnidia of *S. musiva* on leaf spots of poplar species and hybrids may occur on one or both leaf surfaces, and are globose or depressed, embedded in the leaf tissue below the epidermis with protruding ostioles, or with ostioles opening widely, exposing the fruiting layer, and thus resembling acervuli; the walls are thin, dark brown, sometimes indistinct; the width of mature pycnidia varies from 45μ to 150μ. The spores exude in pinkish

[4] This evergreen poplar is reported by Sarasola as a form of *Populus nigra italica* originating in Chile, and named *P. pyramidalis sempervirens*, according to Cornell (*3*, p. 22).

tendrils and are hyaline, cylindric, straight or slightly curved, tapering slightly toward the obtuse tips, usually 2–4 septate, but occasionally continuous, 1, or 5–6 septate. The measurements of spores from pycnidia on leaves were reported by Bier as 17.2μ–51.6μ × 3μ–4μ, and by Thompson as 28μ–54μ × 3μ–4μ. From pycnidia on leaves examined in the present study, the spores measured 17μ–56μ × 3μ–4μ.

On young cankers, particularly those on stems of small succulent shoots, the pycnidia closely resemble those on the leaf spots. On older cankers the pycnidia remain buried in the tissues, with thicker walls. When produced within a lenticel, a pycnidium may develop a thick black stromalike wall, which opens widely in late summer to discharge the spores. The pycnidial cavity may then become filled with brown mycelium and remain sterile thereafter. Frequently several of these pycnidia in a lenticel may coalesce, forming a ring of fruiting bodies just inside the margin of the lenticel. The spores may overwinter within the pycnidia, remaining viable and thus capable of causing early spring infection. The range in length of spores from pycnidia on cankers is approximately that of spores from pycnidia on leaves. Bier reported a range of 21.5μ–55μ, and the writer 24μ–56μ.

The spermagonia have been found only in the autumn on fallen leaves and seem to precede the formation of the perithecia. In the spermagonia numerous hyaline continuous rod-shaped spermatia, measuring about 4μ–6μ × 1.5μ, are produced; these spermatia do not germinate.

Perithecia develop abundantly on fallen overwintered leaves in plantings of hybrid poplar clones, particularly when the trees have been severely infected by *Septoria musiva* during the previous growing season. In Connecticut, leaves collected in May had numerous small black perithecia on both leaf surfaces; these perithecia contained fasciculate asci with eight hyaline, biseriate 1-septate spores typical of *Mycosphaerella populorum*. The spores measured about 15μ–27μ × 4μ–6μ, and were slightly constricted at the septum. They were discharged forcibly from the perithecia and were readily isolated onto a layer of agar in an inverted petri dish. Cultures of these ascospores produced pycnidia and conidia typical of *S. musiva* and resulted in infections when used in stem inoculations.

SUSCEPTIBILITY TESTS

In susceptibility tests, inoculations were made on 202 clones selected by Schreiner for characteristics that would make them desirable for reforestation. They represented 50 combinations of parentage, including cottonwoods, black, and balsam poplars. These hybrid clones, selected from 1947 to 1950, were planted in nursery plots maintained by the U. S. Forest Service at the Agricultural Research Center, Beltsville, Md. Dormant cuttings of the first selections were planted 4 by 4 feet in randomized plots, consisting of 50 trees per clone. Later selections were composed of 25 trees per clone in randomized plots. All plantings were cultivated yearly, and at the end of the first growing season all stems were cut to a 6-inch stump. The vigorous new shoots that were produced from the stumps in the following spring were inoculated in June.

In the experimental plot at the Arnold Arboretum, Jamaica Plain, Mass., five unrooted cuttings of

each clone were planted in nursery rows in the spring of 1948. They were spaced 5 feet apart in rows 10 feet apart. Inoculations were made in July 1949 on the current season's growth of the stems or branches of the 1-year-old trees. Sixty-five of the clones tested were of the parentage *Populus generosa* × *P. jackii*.

In addition to these plantings, a small experimental plot was maintained at Cheshire, Conn. In this planting were 51 clones, 20 trees per clone, representing the early selections made by Schreiner and including those that had shown varying degrees of susceptibility to the canker fungus under natural conditions. This plot served as a source of information concerning the most satisfactory method by which artificial infection may be induced, the time of year most favorable for infection, the plant parts most commonly infected, the development of the disease, and the conditions favoring its spread.

From the first series of inoculations in the Cheshire planting, it was found that infection was easily influenced by the fertility of the inoculum, the weather following the incubation period, and the reaction of each individual tree. Therefore, the inoculation procedure was standardized as much as possible, the stems to be inoculated were carefully selected, and the result of each inoculation was evaluated on the basis of host reaction as well as fungus infection. Infection by *Septoria musiva* spores occurred readily in the succulent tissue of young, vigorously growing shoots. By inserting the inoculum in shallow wounds in such shoots, an immediate reaction by the host and a rapid spread of the fungus in susceptible hosts could be obtained.

Method of Inoculation

To provide sufficient inoculum for each season's inoculations, small cankers with sporulating pycnidia, resulting from inoculation or from natural infection of highly susceptible clones, were collected during the summer and were stored overwinter in a refrigerator at 40° F. About 6 to 8 weeks before inoculations were to be made, conidia from the pycnidia were germinated on malt agar in petri dishes, and 10 or 12 germinating conidia were then transferred singly to tubes of malt agar. As soon as one of these monospore cultures showed abundant sporulating pycnidia, a mass of exuding conidia was placed in 10 cc. of sterile water and a drop of this conidial suspension was poured over a layer of malt agar in a petri dish. When germinated, conidia were transferred individually to tubes of agar. By this method a large number of monospore cultures, with abundant conidial production, could be obtained. Thus, all cultures used in one season's inoculations were derived from the isolation of a single conidium produced in a pycnidium on a canker.

Not all colonies of *Septoria musiva* are fertile; some produce only vegetative mycelium. Also, the fungus grows slowly in culture and its period of fertility is comparatively short. The best results in stimulating the production of sporulating pycnidia were obtained with medium made as follows: To 1 liter of hot Difco malt agar, mixed according to the manufacturer's formula, add 1 gram dihydrogen potassium phosphate, 1 gram magnesium sulfate, and 1 gram peptone, and then sterilize. On this medium, monospore cultures produced abundant mature conidia in 1 to 3 weeks at 25° to 28° C.

All inoculations from which clonal susceptibility was rated were made in wounds on young, vigorously growing shoots. A small triangular section of bark and underlying cambium was cut with a sterile scalpel and turned back from the stem sufficiently to allow the insertion of a small piece of agar with mycelium and pycnidia of the isolate. The bark was then pressed back against the inoculum and the part of the stem inoculated was wrapped with wet cotton and waxed paper. One control on each inoculated clone was made by cutting the stem in the manner used in the inoculations, inserting in the wound a small block of sterile malt agar, and wrapping immediately with wet cotton and waxed paper. The wrappings on all inoculated stems and controls were removed after 2 or 3 days.

In the plantings at Beltsville, Md., the shoots to be inoculated in each clone were carefully selected, in order that stem diameter and vigor of growth might be uniform within the clone. In most clones, the shoots were about three-eighths inch in diameter at the point of inoculation, 18 to 24 inches from the ground. Five or seven inoculations per clone were made in June, each on an individual tree, and a record of the results was obtained the following October. At Cheshire, Conn., most of the inoculations were made in July 1947, and the results were recorded at definite intervals during the following 3 years. On the 1-year-old trees in the planting at Jamaica Plain, Mass., the current season's growth of stems and branches, about one-fourth inch in diameter, was inoculated in July and the results were obtained in October. Four inoculations were made on most of the clones, usually each on an individual tree.

Method of Rating Susceptibility

The inoculated stems were collected 3 or 4 months after inoculation, stacked loosely, and dried at room temperature for at least 48 hours. Each inoculation was tallied into a class descriptive of host reaction to infection, and the visible surface length of each canker was recorded. The specimens were tallied by inoculation number, without regard to parentage or clone, so that each might be considered as an individual.

The classes (fig. 7) that were established as indicative of host reaction were designated as follows:

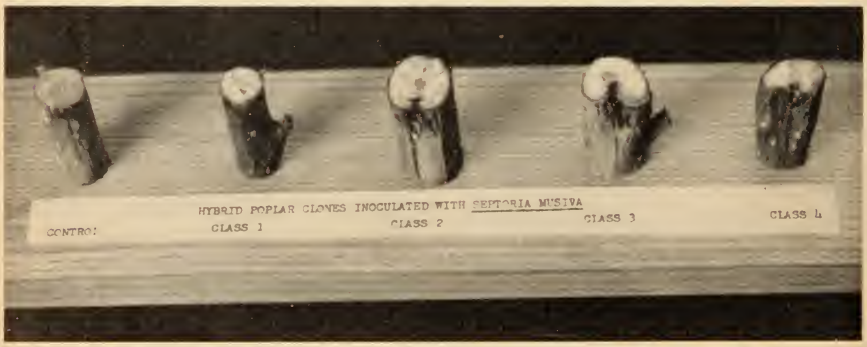

Figure 7.—Cross sections of hybrid poplar clones, showing control (inoculated with sterile malt agar) and class 1 (no infection) to class 4 (highly susceptible) following inoculation with *Septoria musiva*.

Class 1, inoculation wound healed, no lesion; this class included individuals in which no infection occurred because of host resistance, unsuitable inoculum, unfavorable weather conditions, or other external factors that might prevent the development of the fungus.

Class 2, small necrotic lesion around inoculation wound, usually with blackened depressed bark, but no callus, suggesting considerable resistance by the host, or retarded infection from the inoculum.

Class 3, canker with callus at the sides of the inoculation wound, usually with the wood of the wound exposed.

Class 4, conspicuously depressed canker with little or no callus, sometimes with the wood of the stem killed back to the pith, indicating the rapid spread of infection.

Each class number was considered to be an expression of the character of infection resulting from inoculation and was used to obtain a value that would indicate the susceptibility of a clone. The number of individuals in a class was weighted by multiplying by the class number and the sum of the products of all classes represented in a clone was divided by the total number of inoculations in the clone. The weighted averages thus obtained were classified to indicate susceptibility as follows: Weighted average 1.0, no infection: 1.1–1.3, very slight susceptibility; 1.4–2.0, slight susceptibility; 2.1–3.0, moderate susceptibility; 3.1–4.0, high susceptibility. The practical application of this method in rating susceptibility of clones of two hybrids, *Populus deltoides* × *P. trichocarpa* and *P. nigra* × *P. laurifolia*, is given in tables 1 and 2.

For comparison, the clones were rated also into classes, based upon the surface length of cankers in inches, as follows: Class 1, no infection, inoculation wounds approximately ½ inch in length healed; class 2, length of lesion or canker ⅝–1½ inches; class 3, 1⅝–2½ inches; class 4, 2⅝–3½ inches or more. A weighted average for each clone was obtained by multiplying the number of individuals in the class by the class number and dividing by the total number of inoculations in the clone. The detailed application of this method to clones of the parentage *Populus deltoides* × *P. trichocarpa* and *P. nigra* × *P. laurifolia* is given in table 1 in comparison with that of rating by type of canker.

Results of Inoculations

Infection by *S. musiva* following inoculation reproduced the symptoms that result from natural infection. The rapid development of cankers on young shoots during the summer months makes possible an unusually quick evaluation of susceptibility. Also, the prompt healing of all wounds made as controls indicated the ability of actively growing poplar tissue to overcome slight wounding when no parasitic fungus is present.

Early in the study it became evident that not all clones of like parentage reacted to infection in a similar manner. Some clones would show resistance, but others would develop conspicuous cankers. The several clones of the hybrids *P. nigra* × *laurifolia* and *P. deltoides* × *trichocarpa* (table 1) and of *P. generosa* × *jackii* (table 3) illustrate this point particularly. In most of the hybrids, only a small number of clones were tested and therefore the final ratings of susceptibility are summarized according to parentage in tables 2 and 3. These tables also include the total number of inoculations made on all clones of a hybrid and the percentage of infection.

TABLE 1.—*Individual inoculations of hybrid poplar clones classified by type and length of canker resulting from infection by Septoria musiva*

Parentage of Populus hybrids	Clone number	Type of canker [1]					Length of canker [2]				
		Classes				Weighted average [3]	Classes				Weighted average [3]
		1	2	3	4		1	2	3	4	
		No.	*No.*	*No.*	*No.*		*No.*	*No.*	*No.*	*No.*	
deltoides × trichocarpa	OP200	5	2			1.3	5	2			1.3
	201			2	5	3.7		3	4		2.6
	202	4	1	1	1	1.8	4	2		1	1.7
	203	5	1	1		1.4	5	1	1		1.4
	204	4	2	1		1.6	4	3			1.4
	205		4	3		2.4		7			2.0
	206	2	3	2		2.0	2	2	2	1	2.3
	207		1	4	1	3.0		2	2	2	3.0
	208	4	1	2		1.7	4	2	1		1.6
	209	5		1		1.3	5		1		1.3
	210	2	3	2		2.0	2	5			1.7
	211	2	3	1	1	2.1	2	3	2		2.0
	212	3	1	3		2.0	3	2	2		1.8
	213	3	2	1	1	2.0	3	4			1.6
	214	1	1	4	1	2.7	1	3	3		2.3
	215	7				1.0	7				1.0
	216	3		3	1	2.3	3	3		1	1.8
	217	4	1			1.2	4	1			1.2
	218	2	2	2	1	2.3	2	3	2		2.0
	219	3	1	2	1	2.1	3	2	2		1.8
	220	1	1	4	1	2.7	1	3	3		2.3
nigra × laurifolia	1	2	3	1		1.8	2	2	2		2.0
	2	1		3	8	3.5	1	2	6	3	2.9
	3	2	2	4	1	2.4	2	2	5		2.3
	4	1	1	3	7	3.3	1	1	6	4	3.1
	5		2	3	7	3.4		2	5	5	3.2
	6			2	7	3.8		2	3	4	3.2
	7			2	7	3.8		2	7		2.8
	8	9				1.0	9				1.0
	279	4	2		1	1.7	4	2		1	1.7
	280	1	5	1		2.0	1	4	2		2.1
	281	4	3			1.4	4	2	1		1.6
	282	3	4			1.6	3	4			1.6
	283	3			4	2.7	3	2	2		1.8
	284				3	4.0				3	4.0
	343	2			5	3.1	2	1	3	1	2.4

[1] Type of canker: Class 1—no lesion; class 2—small lesion around partially healed inoculated wound; class 3—calloused canker with exposed wood; class 4—depressed canker with little or no callus.

[2] Length of canker—surface length in inches: Class 1—no lesion; class 2—length ⅝ to 1½; class 3—1⅝ to 2½; class 4—2⅝ to 3½.

[3] Weighted average was obtained by multiplying the number of individuals in a class by the class number and dividing the sum of the products of all classes in the clone by the total number of inoculations in the clone.

TABLE 2.—*Susceptibility of hybrid poplar clones to Septoria musiva, based on type of cankers from inoculations at Beltsville, Md., and Cheshire, Conn.*

Parentage of Populus hybrids (female listed first)	No infection	Susceptibility [1]				Inoculations	
		Very slight	Slight	Moderate	High	Total	Infected
	Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Percent*
angulata × berolinensis	1	1	2	2		44	38
angulata × deltoides		1	2			19	31
angulata × incrassata		1	1		1	23	47
augulata × nigra plantierensis	2		1		1	28	46
angulata × trichocarpa			2	8		70	72
angulata × volga poplar	1	1	1	1		28	21
angulata × unidentified cottonwood	1		1			14	14
berolinensis × serotina	2					10	0
candicans × berolinensis		1	1	2	3	50	82
charkoviensis × berolinensis				1	1	18	83
charkoviensis × caudina	2	4	3	2	1	90	40
charkoviensis × deltoides	2		3			42	23
charkoviensis × incrassata		1			2	25	76
charkoviensis × nigra plantierensis	1	2	1	2		41	36
charkoviensis × robusta		1		1		12	33
charkoviensis × trichocarpa			1	3	2	45	82
charkoviensis × volga poplar	1					7	0
charkoviensis × cottonwood			1	1		17	65
deltoides × berolinensis	3	1	1	1		35	26
deltoides × caudina	2	1	1	4		54	61
deltoides × grandidentata	1					7	0
deltoides × incrassata				1		7	100
deltoides × nigra plantierensis			2	2		24	54
deltoides × trichocarpa	1	3	8	8	1	143	58
deltoides × volga poplar	2	2				28	7
fremontii × trichocarpa				1		7	57
maximowiczii × berolinensis				3	5	78	91
maximowiczii × caudina					1	9	100
maximowiczii × nigra plantierensis					2	21	95
maximowiczii × trichocarpa					2	15	100
nigra × eugenii				1		5	100
nigra × laurifolia	1		5	2	7	123	83
nigra × nigra italica	1				1	11	45
nigra × trichocarpa		2	1		1	31	48
nigra baatanicorum vitrum × nigra plantierensis	1		1			12	8
nigra baatanicorum vitrum × trichocarpa	1				1	14	64
nigra baatanicorum vitrum × volga	2		2			26	23
nigra betulifolia × trichocarpa	1		3	3	1	54	63
nigra betulifolia × volga poplar		1	1	1		19	53

See footnote at end of table.

TABLE 2.—*Susceptibility of hybrid poplar clones to Septoria musiva, based on type of cankers from inoculations at Beltsville, Md., and Cheshire, Conn.*—Continued

Parentage of Populus hybrids (female listed first)	No infection	Susceptibility [1]				Inoculations	
		Very slight	Slight	Moderate	High	Total	Infected
	Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Percent*
petrowskyana × caudina		2	2	1	1	43	56
rasumowskyana × caudina	1				1	16	56
rasumowskyana × incrassata				1		7	71
rasumowskyana × nigra plantierensis	1		1			14	28
rasumowskyana × trichocarpa					1	7	100
sargentii × berolinensis rossica			1	1	1	26	73
sargentii × nigra italica		1				7	14
sargentii × sargentii	1					7	0
sargentii × simonii	1					7	0
simonii × berolinensis		1	1	1	1	28	61
Totals	33	27	50	54	38		

[1] Classes of susceptibility were derived from weighted averages of individual clones obtained by multiplying the number of individuals in a class by the class number and dividing the sum of the products of all classes in the clone by the total number of inoculations in the clone. Weighted average 1.0, no infection; 1.1–1.3, very slight susceptibility; 1.4–2.0, slight susceptibility; 2.1–3.0, moderate susceptibility; 3.1–4.0, high susceptibility.

The rating of susceptibility by type of canker, as shown in tables 1, 2, and 3, proved to be the most satisfactory method of analysis. In the susceptibility classes the retarding of infection by the production of callus is taken into account. Rating by surface length of cankers in the low susceptibility classes sometimes resulted in a weighted average that was identical or similar to that derived from rating by type of canker. In the moderate susceptibility class, however, the cankers might measure longer on the surface than those in the high susceptibility class, but the latter would extend deep into the wood and increase in severity during successive growing seasons. Not all clones included in the moderate and high susceptibility classes in tables 2 and 3 are recommended for propagation, even though the vigorously growing trees may live for some years after large cankers have formed on the trunks. The clones listed as not infected and very slightly susceptible were retested by inoculations in another locality and the same ratings were obtained. They should, therefore, prove to be of value in reforestation and breeding projects.

In the analysis of the clones, certain parental variations in susceptibility are evident. The hybrid clones with the clone of *Populus maximowiczii* as the female parent proved highly susceptible and infections resulted from 91 to 100 percent of the inoculations on the 4 hybrids. In other hybrids in which more than 30 clones were tested, those in which the female clones of *P. angulata*, *P. charko-*

TABLE 3.—*Susceptibility of poplar clones to Septoria musiva, based on type of cankers from inoculations at Jamaica Plain, Mass.*

Populus species and hybrids	No infection	Susceptibility [1]				Inoculations	
		Very slight	Slight	Moderate	High	Total	Infected
	Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Percent*
generosa × jackii	1	3	31	29	1	247	85
trichocarpa hastata [2]				3		12	91
trichocarpa × nigra				1		4	50
trichocarpa × koreana			1			4	50
trichocarpa × tacamahaca				1		3	100
generosa	1					4	0
jackii			1			4	50
maximowiczii [2]	1			1		7	43
maximowiczii × nigra	1					4	0
nigra		1				4	25
nigra italica	1					2	0
berolinensis			1			4	50
robusta		1				4	75
koreana				1		4	100

[1] Classes of susceptibility were derived from weighted averages of individual clones obtained by multiplying the number of individuals in a class by the class number and dividing the sum of the products of all classes in a clone by the total number of inoculations in the clone. Weighted average 1.0, no infection; 1.1–1.3, very slight susceptibility; 1.4–2.0, slight susceptibility; 2.1–3.0, moderate susceptibility; 3.1–4.0, high susceptibility.

[2] Open pollinated.

TABLE 4.—*Susceptibility of hybrid poplar clones to Septoria musiva in relation to the species and sex of the parents*

Species of Populus	Number of clones	No infection	Susceptibility			
			Very slight	Slight	Moderate	High
Female parent:		*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
angulata	32	16	16	28	34	6
charkoviensis	39	15	20	23	25	17
deltoides	45	20	15	27	36	2
nigra	22	9	9	27	14	41
maximowiczii	13	0	0	0	23	77
Male parent:						
berolinensis	33	12	12	15	30	31
caudina	29	17	24	21	24	14
deltoides	8	25	13	62	0	0
incrassata	8	0	25	13	25	37
nigra plantierensis	20	25	15	25	20	15
trichocarpa	55	5	9	27	42	17
volga	16	37	25	25	13	0

viensis, and *P. deltoides* were crossed with the male clone of *P. trichocarpa* showed high susceptibility. All clones of the hybrids *P. angulata* × *P. trichocarpa* and *P. charkoviensis* × *P. trichocarpa* were susceptible, with 72 and 82 percent infection, respectively. Of the 21 clones of the hybrid *P. deltoides* × *P. trichocarpa*, one showed no infection and 17 ranged from slight to high susceptibility, with a total infection of 58 percent. Hybrids with the so-called Volga poplar as male parent tended to be resistant, but those with the clone of *P. berolinensis* as male parent were more susceptible. These variations may indicate disease tendencies inherent in the species of the parent clones, or related to the sex factor, but the limited data from the present study merely suggest the possibility of such tendencies. The data in table 4, however, summarize these variations in susceptibility among the clones of hybrids with certain species as male or female parent.

SUSCEPTIBILITY OF CLONES TO NATURAL INFECTION

Clonal variations in susceptibility to natural infection were observed in the 51 hybrid clones tested at Cheshire, Conn., and were compared with susceptibility ratings obtained from inoculations. From stem inoculations made in July 1947, one clone failed to become infected and three were very slightly susceptible. During the two following years, leaf infection became severe on all clones and ascospores were produced in abundance on fallen leaves, thus increasing the amount of inoculum in the planting. Early in 1950 before growth started, all trees in the plot were cut down and the shoots that developed from the stumps were not inoculated with the *Septoria*. By the latter part of July, septoria leaf spots and cankers from natural infection were abundant on 24 of the clones, all of which had been rated previously as moderately or highly susceptible. In June 1951, 15 additional clones showed stem infection. The 12 uninfected clones included the 4 that had showed little or no infection from inoculations and the 8 that had been rated as slightly susceptible. During the summer, the leaves of all clones were heavily infected and stem infection occurred on occasional trees of the resistant clones. The cankers thus formed developed very slowly and many healed over entirely. In contrast, girdling cankers on the highly susceptible clones killed many stems and all trees of some clones.

The rating of susceptibility by means of inoculations on young shoots was closely duplicated by natural infection of shoots of similar age in the same locality, but even the more resistant clones showed considerable infection when an abundance of inoculum was present in the planting.

Observations on the reaction to natural infection by other hybrid clones that failed to become infected, as listed in table 2, have not yet been sufficient to indicate whether their tendency will be maintained during continued propagation and under various climatic and environmental conditions. All of them are susceptible to leaf infection and might develop a few cankers when conditions in the plantings are favorable for the spread of the fungus. None of the clones tested, therefore, can be considered immune.

Natural infection of some of the clones listed in table 3 was observed

in a planting at Rochester, Minn.,[5] established in 1949. At present many of the trees are heavily cankered or have been killed by canker insects, and unfavorable environment. The possible effect of natural infection by *Septoria musiva* cannot now be compared with the susceptibility rating derived from inoculations. However, one of the clones of *Populus trichocarpa hastata*, rated from inoculations as moderately susceptible, showed numerous cankers resembling those caused by *S. musiva*. Another clone of the same host, representing a tree of superior growth from the State of Washington, and included in Pauley's collection, had young cankers with typical *Septoria* pycnidia. This clone has not been tested by inoculation. In the same planting, a clone of *P. trichocarpa* from Idaho, probably of the variety *hastata*, was similarly infected, and *S. musiva* was isolated in culture from the outer margin of the cankers. This indicates that clones of this host may be susceptible to natural and artificial infection, at least outside the native range of the species. Leaf spots caused by *S. musiva* have been reported on this host in its native range, but no reference to stem or branch infection has been made.

RELATION OF SECONDARY FUNGI TO INFECTION BY *SEPTORIA MUSIVA*

The bark and wood of stems infected by *Septoria musiva* readily become attacked by secondary fungi. On cankers, fruiting bodies of these fungi are frequently more abundant and conspicuous than those of the *Septoria*, and therefore they may be more easily isolated from wood tissue in the infected area.

On hybrid poplars that are highly susceptible to *Septoria*, a species of *Phomopsis* frequently attacks the newly infected area during the latter part of the growing season, and its pycnidia become abundant on the canker during the following season. No perfect stage of this fungus has been found in nature or in culture, and therefore the species has not been identified. Tissue cultures from the area produce the *Phomopsis* rather than the *Septoria*, thus confusing the identification of the causal organism. Inoculations with isolates of the *Phomopsis* were made in wounds on healthy stems and lateral branches, but no infection occurred. The *Phomopsis* very rarely infects twigs that have been killed back from infection by some other fungus or from winter injury. Its spores are readily isolated from buds and lenticels of dormant cuttings from plots in which the fungus is known to be present. On more resistant clones, which react to septoria infection by forming callus, the *Phomopsis* may be present for a short time following infection, but the growth of callus gradually prevents its further development.

Two species of *Cytospora*, *C. chrysosperma* and *C. nivea* Sacc. (*18*), are frequent on twigs and branches of hybrid poplars following winter injury and on stems or young shoots that have been attacked by insects or killed back by girdling cankers, such as those caused by septoria infection. They also cause cankers on trees of vari-

[5] The planting of clones of hybrid poplars and those from species trees selected for superior growth was established at Rochester, Minn., by the Mayo Forestry and Horticulture Institute, in cooperation with the Department of Forestry, University of Minnesota.

ous species of poplar that have been weakened by unfavorable soil or other environmental conditions. Usually fruiting bodies of *Cytospora* are not formed directly on the area in which infection by *Septoria* took place, but appear beyond the outer margin of the primary canker. *Cytospora chrysosperma* may develop fruiting bodies in abundance on specimens of stems having *Septoria* cankers when brought into the laboratory, even though it was not evident when the specimens were collected in the nursery. Old calloused septoria cankers may have a few fruiting bodies of the *Cytospora* and it is commonly isolated from tissue cultures of such cankers. *Cytospora nivea* fruits abundantly on stems weakened by winter injury. Its perfect stage, *Valsa nivea* Hoff. ex Fr., develops during the second season after the death of the stems, and also on stems weakened or killed by *Septoria*. *Valsa sordida* Nits., the perfect stage of *C. chrysosperma*, seems to be less commonly found, but the two *Cytospora* species, and their perfect stages, may occur together on the same dead stem.

Other fungi have been found frequently in old cankers, or on recently killed stems of the hybrid clones that are most susceptible to Septoria infection. These include species of *Dothiorella*, *Diplodia*, *Fusarium*, *Tympanis*, and *Cenangium*, and are less prevalent than the species of *Cytospora* and *Phomopsis*. The abundance of their fruiting bodies on a canker is no indication that they are parasitic, but rather suggests that the affected area should be examined for the presence of *Septoria* pycnidia and that tissue cultures from the margin of the canker should be made. None of these fungi has been found to be the primary cause of cankers on the hybrids studied. Miscellaneous ascomycetes and imperfect fungi, as well as wood-rotting fungi, follow all these secondary fungi, bringing about a rapid decay of the diseased trees. This sequence of attack causes extensive destruction of stems or trunks weakened or girdled by septoria infection.

The extreme vigor of some of the highly susceptible clones enables them to remain alive, even though large trunk cankers are present. On such cankers, secondary fungi develop rather slowly, but usually can be isolated in cultures from wood tissue. Other clones, particularly one of the hybrid *Populus charkoviensis* × *P. trichocarpa*, are highly susceptible but less vigorous in growth. This one clone has been rapidly attacked by secondary fungi following infection by *Septoria*, and the trees have been killed. Another clone of this same hybrid is similarly susceptible, but has not been so severely attacked by secondary fungi. Trees of this clone are weakened by the infection but continue to maintain good growth for some years. The death of stems or trunks of hybrid poplars, therefore, may be initiated by septoria infection, but their decay may be considerably hastened by secondary fungi.

CONTROL MEASURES

Septoria canker is difficult to control after it becomes established in a planting. Therefore all measures that will tend to prevent or control infection in a new planting should be employed. The selection and propagation of hybrid poplar clones that have proved to be resistant to natural and artificial infection is of primary importance. No clone among those already tested can be considered immune. The wide vari-

ation in susceptibility of clones of one parentage presents difficulties in establishing a select group of clones that would be adapted to propagation under diverse climatic and soil conditions. The group designated in table 2 as not infected has shown high resistance in the eastern United States, but might prove less resistant elsewhere. The resistance of this group, however, indicates the possibility and importance of a careful selection of clones representing various combinations of parent species. Even though only resistant clones are selected, they should be planted in randomized plots or mixed groups, particularly when grown under climatic or soil conditions unlike those in which their resistance was tested. This will counteract the effect of losses from infection brought about by changes in susceptibility that may occur under new environment.

Stem infection is preceded by leaf infection in plantings established from unrooted cuttings. Because of the prevalence of leaf spots caused by *Septoria musiva* on native poplars, leaves of all exotic and hybrid poplars within the reach of windborne spores may readily become infected. The young shoots of resistant clones may develop lesions occasionally during the first 2 or 3 years of growth, particularly if moisture conditions are favorable for the infection of the dense juvenile foliage near the ground. Wide spacing combined with good aeration and drainage will tend to counteract this condition. After the trees are over 10 feet tall, the possibility of stem infection decreases as the lowest branches die from shading and the distance between discharged ascospores from fallen overwintered leaves on the ground and the newly developing leaves becomes greater. Leaves on older trees of hybrids that retain numerous low branches may have leaf spots, but if a clone is highly resistant, the trees will remain free from twig and stem cankers.

In stool beds, septoria leaf spots frequently are abundant and the young succulent shoots developing from the stumps are particularly susceptible to infection. Spraying with a 4–4–50 Bordeaux mixture or any other fungicide commonly used to control leaf spots will control the spread of leaf and stem infection. The first application should be made as soon as the leaves develop in the spring, and should be followed by 2 or 3 additional applications during the growing season at intervals of 2 weeks. Spores of the *Septoria* may become lodged in the lenticels and in the resinous coating of the buds. The fungicides used as a spray will not penetrate sufficiently to kill such spores and they remain viable on dormant cuttings during storage. They may germinate when the cuttings are planted, thus starting infection in new plantings.

Cuttings from infected stool beds should be surface sterilized before they are shipped into localities where susceptible clones are growing or where the disease has not occurred. Satisfactory sterilization may be obtained by dipping dormant cuttings in solutions of certain mercury compounds without subsequent rinsing in water (*22*, *23*). The mercury residual from some fungicides causes phytotoxicity if the treated cuttings are stored for several weeks before planting. Solutions of Ceresan M and Semesan have proved to be effective in sterilizing cuttings of hybrid poplar clones. Dormant cuttings dipped in these solutions, stored 2 weeks following treatment, then planted in the greenhouse, produced normal shoots and roots. However, in nursery plantings of cuttings similarly

treated and stored, only the cuttings dipped in Semesan showed satisfactory survival and growth (*9*).

SUMMARY

Canker caused by the fungus *Septoria musiva* is an important and destructive disease of hybrid poplars, with black, balsam, and cottonwood parentage, in the United States. It has been reported also in Canada and Argentina. The fungus causes leaf spots on native poplars, but these species are resistant to stem infection in their natural range. Clones of introduced species are frequently susceptible. Variations in susceptibility to the disease have been evident in plantings of hybrid poplar clones that were selected as particularly desirable for pulpwood, reforestation, and soil conservation.

Leaf infection precedes twig or stem infection and in newly established plantings it may be caused by windborne spores produced in pycnidia in leaves of nearby native poplars. *Septoria* spores may also be carried on dormant cuttings in the lenticels or in the resinous coating of the buds. In older plantings the leaves become infected in early spring by conidia from pycnidia that have overwintered in cankers or by ascospores of the perfect stage, *Mycosphaerella populorum*, that are formed in perithecia in fallen overwintered leaves. Conidia from pycnidia on leaf spots or cankers infect young stems through lenticels or wounds. Infection may also spread into the stem from infected petioles. Cankers develop rapidly on highly susceptible hosts, and young stems may be girdled in one growing season. More resistant hosts form callus tissue that retards or prevents the spread of the fungus. In hosts that are very slightly susceptible only a few small necrotic lesions are formed and these usually heal during the growing season.

On cankers, the pycnidia of *S. musiva* are usually rare and inconspicuous. Secondary fungi soon infect the tissue killed by septoria infection and the rapid development of their fruiting bodies tends to prevent the fruiting of the more slowly growing *Septoria*. Their presence on the cankers may make it difficult to determine the primary cause.

Because of the wide variation in host reaction to stem infection among the hybrid poplar clones, no distinctive canker can be described as peculiar to *Septoria musiva*. The occurrence of the *Septoria* pycnidia on a canker, or the isolation of the fungus in cultures from wood tissue at the advancing edge of the canker, are the only means by which an exact determination can be made.

Young shoots of hybrid poplar clones were successfully inoculated in early summer with *Septoria musiva* through stem wounds, with sporulating pycnidia from malt agar cultures as the inoculum. At the end of the growing season the result of each inoculation was tabulated into a class representing the effect of infection and the reaction of the host. Weighted averages from this analysis were separated into classes indicating the susceptibility of the host. On this basis, among 202 clones tested, 33 showed no infection, 27 were very slightly susceptible, and 38 were highly susceptible. Similar results were obtained by using the superficial length of lesions or cankers as the basis for analysis and rating susceptibility. Variations in susceptibility to natural infection were observed in 51 of the tested clones. Susceptibility of these individual clones corresponded with their ratings based on inoculations. Among

65 clones of the parentage *Populus generosa* × *P. jackii* tested by inoculations, one was not infected and 61 showed slight to high susceptibility. Clones of *P. trichocarpa hastata*, native in the northwestern United States, were susceptible to natural infection in Minnesota and to artificial inoculation in Massachusetts.

The most important and satisfactory method of controlling the canker is the use of hybrid poplar clones that have proved to be resistant when tested by natural infection or by artificial inoculations. Even though only resistant clones are selected, they should be planted in randomized plots or mixed groups, particularly when grown under climatic or soil conditions unlike those in which their resistance was tested. This will counteract the effect of losses from infection brought about by changes in susceptibility that may occur under new environment. Precautionary measures that will tend to retard infection in a new planting include the surface sterilization of dormant cuttings, the spraying of stool beds with a fungicide to prevent leaf infection, and a spacing of trees that will provide adequate aeration near the ground, thus preventing high humidity that would be particularly favorable for the infection of leaves and stems.

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